HEMOSTATIC COMPOSITIONS AND DEVICES

FIELD OF THE INVENTION

The present invention relates to hemostatic compositions suitable for use in hemostatic devices that in turn are suitable for applying flowable hemostatic compositions to a site requiring hemostasis, to such hemostatic devices containing such compositions disposed therein and to methods of making such hemostatic compositions and devices.

BACKGROUND OF THE INVENTION

Gelatin-based hemostats, both in solid sponge or powder form, are commercially available and are used in surgical procedures. Gelatin powder, when mixed with fluid, can form a paste or slurry that is useful as a flowable, extrudable and injectable hemostat for diffuse bleeding, particularly from uneven surfaces or hard to reach areas. The conventional slurry is prepared at the point of use by mechanical agitation and mixing of the powder and liquid to provide uniformity of the composition. The paste then is placed into a delivery means or applicator, e.g. a syringe, and applied to the wound.

The main disadvantage of this approach is the need to mix the powder with the liquid, knead it into a paste and back-fill it into the delivery device of choice, all at the time of need and at the point of use. The manipulations are time consuming and potentially can compromise the sterility of the delivered product. Thus, a need exists for a sterile, flowable, moldable hemostatic composition that is ready to use at the point of use or can be prepared with minimal manipulation and without risk of compromising the sterility of the product.

It would be desirable if a hemostatic device, e.g. a delivery means such as a syringe or other applicator, would be pre-filled with a hemostatic composition and instantly available to the surgeon at the point of use without need for further manipulation. The hemostatic composition pre-filled in the device or applicator should be sterile and flowable and should require minimum preparation time and minimal force when extruded or injected through the delivery means at the point of use. The compositions of the present invention provide such properties and pre-filled devices.

SUMMARY OF THE INVENTION

The present invention is directed to hemostatic compositions suitable for use in preparing hemostatic devices that in turn are suitable for use in applying flowable hemostatic compositions and comprising a substantially homogenous, sterilized hemostatic composition disposed therein, where the composition comprises a continuous, biocompatible liquid phase, a solid phase comprising porous or non-porous particles of a biocompatible polymer suitable for use in hemostasis and which are substantially insoluble in the liquid phase, and a discontinuous gaseous phase comprising a biocompatible gas. The continuous liquid phase comprises the solid particulate phase and the discontinuous gaseous phase substantially homogenously dispersed there through. The ratio of the liquid phase, the solid particulate phase and the discontinuous gaseous phase is effective to provide the composition with hemostatic properties, both prior to and after sterilization. Compositions of the present invention may be prepared well in advance of the time of use and need not be prepared at the point of use, yet they maintain physical properties effective to provide flowability, extrudability or injectability at the point and time of use. The present invention also includes methods of making the hemostatic compositions, medical

devices containing the compositions disposed therein and methods of making such devices.

DETAILED DESCRIPTION OF THE INVENTION

Both sterilized and unsterilized compositions of the present invention contain solid, porous or non-porous particles of a biocompatible polymer suitable for use in hemostasis, a biocompatible liquid and a biocompatible gas as its three necessary components. The particles, liquid and gas are combined and mixed under conditions effective to provide a substantially homogeneous hemostatic composition comprising a continuous liquid phase having the solid polymer particles and a discontinuous gas phase homogenously dispersed there through. The amount and average diameter of particles contained in the composition and the relative amounts of the solid, liquid and gaseous phases is effective to provide the composition with hemostatic and physical properties, as described herein below.

The hemostatic composition so formed is a hemostatic paste, or slurry, that exhibits improved properties of flowability, extrudability and/or injectability when compared to flowable hemostatic compositions of similar liquid/particle composition but that do not contain a gaseous phase. Compositions of the present invention may be prepared, filled into a medical device, such as a syringe or other known applicators used to dispense flowable hemostatic compositions, and sterilized by ionizing irradiation, well in advance of the time of their intended use. The compositions further may include additives to facilitate the preparation of the composition, enhance physical and mechanical properties, enhance the hemostatic properties of the composition or provide antimicrobial properties.

As used herein, "continuous" and "discontinuous" are used in the ordinary meaning of those words in the context of standard nomenclature used to define and

describe dispersions. For example, when combined and mixed with the continuous liquid phase, the volume of biocompatible gas added to the liquid phase is disrupted by mixing so as to form the discontinuous, i.e. dispersed, gaseous phase comprising pockets or isolated bodies of gas.

As used herein, "substantially homogenous" denotes that physical state of the compositions or pastes where the solid and/or gaseous phases are uniformly dispersed throughout the continuous liquid phase such that the ratio of solid:gas:liquid and the density of any portion or cross-section of the composition or paste are substantially the same.

As used herein, "sterile" means substantially free of living germs and/or microorganisms and as further recognized and described by governmental standards pertaining to compositions and medical devices described and claimed herein.

As used herein, "hemostatic", or "hemostatic properties", means the ability to stop or minimize bleeding, as one skilled in the art of hemostasis would understand those terms to mean, as further exemplified in the examples of the specification.

As used herein, "Peak Expression Force" is the peak force value required to extrude compositions from a pre-filled 10cc Becton Dickinson (BD) luer syringe fitted with a 14 gauge angiocatheter tip, as described in the examples of the specification.

A variety of biocompatible natural, semi-synthetic or synthetic polymers may be used to prepare the solid particles used in compositions of the present invention. The polymer selected must be substantially insoluble in the liquid chosen for the particular composition. Preferably, water-insoluble biodegradable polymers that provide mechanical, chemical and/or biological hemostatic activity are used. Polymers that may be used include, without limitation, proteins and polysaccharides. Polysaccharides that may be used include oxidized cellulose, chitosan, chitin, alginate, oxidized alginate and oxidized starch. The biocompatible polymer used to prepare the particles

preferably is a cross-linked or denatured protein, such as gelatin, collagen, fibrinogen or fibronectin. A preferred gelatin powder is Surgifoam® hemostatic gelatin powder, available from Johnson & Johnson Wound Management, a division of Ethicon, Inc. Surgifoam® powder is a partially cross-linked gelatin powder prepared by milling gelatin sponge into particles having an average diameter of from about 40 microns to about 1200 microns, more preferably from about 100 microns to about 1000 microns, as determined by laser diffraction.

Compositions of the present invention comprise a continuous liquid phase in which the solid particles and gaseous phase are dispersed. Depending upon the particular medical device and use thereof, the liquid may be aqueous or non-aqueous. Preferably, the liquid phase is aqueous. Aqueous liquids may include, without limitation, biocompatible aqueous solutions, such as calcium chloride and saline. More preferably, the liquid phase comprises saline. The liquid phase and solid particulate phase are present in relative amounts effective to provide a paste, or slurry, suitable for use in providing hemostasis. Excessive dilution of the solid particulate phase, although beneficial to further reduce the peak expression force, will detrimentally affect the hemostatic properties of the material and therefore is not desired. The weight ratio of solid particles to liquid generally is from about 1:2 to about 1:12. A preferred weight ratio of the solid gelatin particles to saline is from about 1:3 to about 1:6. A more preferred weight ratio of the solid gelatin particles to saline is about 1:5.

Any biocompatible gas may be used to prepare compositions of the present invention including, but not limited to, air, carbon dioxide, nitrogen, xenon or argon. Preferably an inert gas such as argon or nitrogen is used. Air, nitrogen and argon are sensitive to ultrasound and may provide a means to locate the composition once injected in the body. Similarly, as xenon is radio-opaque, using xenon also may provide a means to locate the composition once placed in the body. In addition, as

carbon dioxide lowers pH, selection of carbon dioxide may enhance antimicrobial properties of the compositions. The gas must be combined and mixed with the continuous liquid phase until it is uniformly dispersed throughout the liquid phase so as to form a discontinuous, gaseous phase substantially homogenously dispersed in the continuous liquid phase. The homogenous dispersion of the gas phase in the liquid phase provides the composition with improved physical properties relating to flowability, extrudability and injectability, as described herein. Such improved properties are characterized by way of physical measurements of the compositions, including density and peak expression force, both prior to and after irradiation of the compositions during sterilization.

The relative concentration of the three major components of the compositions of the present invention and the substantially homogenous nature of such compositions are key in providing both hemostatic and physical properties to the compositions. The solid particles, liquid phase and gaseous phase generally will be present in compositions of the present invention at a ratio of from about 1:2:1 to about 1:12:13, based on weight:volume:volume (g:ml:ml). Preferably the ratio will be from about 1:4:1 to about 1:8:9. More preferably the ratio will be about 1:5:3. The density of compositions of the present invention will be from about 0.9 g/ml to about 0.3 g/ml, more preferably from about 0.8 g/ml to about 0.6 g/ml.

Compositions of the present invention include compositions described herein that are sterile, in that they have been irradiated with a level of, e.g. ionizing irradiation. Such irradiation may include e-beam or gamma irradiation. The level of irradiation and conditions of sterilization, including the time that the compositions are irradiated, are those that provide sterile compositions, as defined herein. Once having the benefit of this disclosure, one skilled in the art will be able to readily determine the level of irradiation necessary to provide sterile compositions.

The hemostatic compositions may further comprise effective amounts of one or more additives or compounds including, but not limited to, antimicrobial agents, foaming agents, foam stabilizers, surfactants, antioxidants, humectants, wetting agents, lubricants, thickeners, diluents, irradiation stabilizers, e.g. radical scavengers, plasticizers, and stabilizers. For example, glycerol may be added to enhance the extrudability or injectability of the composition. When utilized, glycerol may be present in the compositions at from about 0% to about 20% by weight, based on the weight of the liquid phase. Preferably, the composition may comprise from about 1% to about 10% by weight of glycerol, based on the weight of the liquid phase. More preferably, the compositions may comprise from about 1% to about 5% by weight of glycerol, based on the weight of the liquid phase.

In addition, quaternary amines may be used to provide enhanced properties to the compositions. For example, benzalkonium chloride, Polybrene or Onamer M may be used at levels up to about 1 percent by weight, based on the weight of the liquid phase. Preferably, benzalkonium chloride is used at levels of from about 0.001% to about 0.01 % by weight, based on the weight of the liquid phase. More preferably, the compositions may comprise from about 0.002 to about 0.006% by weight benzalkonium chloride, based on the weight of the liquid phase. It is believed that the quaternary amines may serve multiple functions, acting as an antimicrobial agent, a foaming agent, a radical scavenger and as a heparin neutralizer.

Such hemostatic compositions may further comprise heparin neutralizers, procoagulants or hemostatic agents, such as thrombin, fibrinogen, fibrin, Factor Xa, or Factor VIIa. By "effective amount", it is meant that amount necessary to provide to the compositions those properties for which the additive is being added. The effective amount also is limited by the maximum amount that may be added without causing detrimental biological affects.

Compositions of the present invention are particularly advantageous for use in hemostatic compositions where additives that are sensitive to irradiation, are utilized. For example, thrombin, in an aqueous solution, has been found to lose all procoagulant activity when exposed to sterilization irradiation. In contrast, thrombin retained approximately 40% of its original enzymatic activity and all of its hemostatic activity after sterilization when formulated in compositions according to this invention, as shown in Example 9. While bovine thrombin is exemplified herein, human-derived thrombin also may be used in compositions of the present invention.

Medical devices in which the hemostatic compositions of the present invention may be utilized include any device currently being used to apply a flowable or injectable hemostatic paste or slurry to a site, or wound, requiring hemostasis. The site requiring hemostasis may be the result of an injury or a surgical procedure. Examples of devices or applicators include syringes such as Becton Dickinson or Monoject luer syringes. Other devices are disclosed in detail in United States Patent No. 6,045,570, the contents of which are incorporated by reference in their entirety.

In one embodiment for making compositions of the invention, a substantially homogenous paste is prepared by first mixing the particles with the liquid to form a uniform paste. The liquid may include effective amounts of additives dissolved therein as described above. The gas then is incorporated into the paste and mixed until it is homogenously dispersed throughout the paste, thus providing a discontinuous gaseous phase dispersed throughout the continuous liquid phase. Mixing may be accomplished by extrusion or by mixing in a confined space under conditions effective to provide a uniform dispersion of the solid particles and gas into the liquid phase. Substantially homogeneous dispersion of the gas into the paste or slurry is important in providing the compositions with their desired properties. If the gaseous phase is not homogeneously dispersed through out the paste, the density of the combined gaseous phase and paste

will not be effective to provide the compositions with adequate peak expression force both prior to and after sterilization of the composition. When prepared in this manner, a preferred volume ratio of gas to paste is from about 1:10 to about 2:1. A more preferred volume ratio of gas to paste is from about 1:5 to about 1:1. An even more preferred volume ratio of gas to paste is from about 1:2 to about 1:1.

Alternately, a mixer, e.g. a double planetary mixer, may be utilized in making compositions of the present invention. The liquid is added to the mixer. The liquid may include effective amounts of additives dissolved therein prior to addition of particles or the gas to the solution. For example, a saline solution containing glycerol and benzalkonium chloride may be prepared and then added to the mixer. A source of gas is provided to the mixer whereby a first portion of the gas may be added to the liquid solution. The mixture of gas and liquid is mixed to disperse the gas in the liquid phase, forming a foam-like consistency. The balance of the gas and the solid particles are added to the mixer over time with continuous mixing until all ingredients have been added. The mixing is continued until such time as a substantially homogenous composition is formed containing the solid particles and gaseous phase uniformly dispersed throughout the continuous liquid phase.

The hemostatic compositions prepared as above are transferred into a medical device as described above and the device containing the hemostatic composition is sterilized, preferably by ionizing radiation. More preferably, sterilization is by gamma irradiation as exemplified herein.

While the following examples demonstrate certain embodiments of the invention, they are not to be interpreted as limiting the scope of the invention, but rather as contributing to a complete description of the invention.

Examples:

Samples prepared in the Examples below were tested for peak expression force as determined using a Chatillon TCD 200, using a 50-lb load cell [DFG 550] at a speed of 2 inches/min. An in-dwelling catheter sheath (size 12-14 gauge) was attached to the sample syringe to be tested. The syringe then was inserted into a holding apparatus, which then was loaded onto the test instrument. The peak expression force was noted.

Example 1:

A total of ten samples were prepared as follows. One gram of dry Surgifoam® powder was placed in a plastic container and mixed with 4 ml of saline. The container was capped and the contents were shaken until a substantially homogenous paste of uniform consistency was obtained. The paste was formed into a cylindrical shape and placed into a 10cc BD polypropylene disposable luer syringe. The syringes were then capped and five of the filled syringes were sterilized by gamma irradiation at a dose of 25 kGy. The Peak Expression Force was determined and presented in Table 1. Unsterilized samples are designated as 1a and sterilized samples are designated as 1b.

At total of 10 samples were prepared as follows. 1 gm of dry Surgifoam® powder was placed in a plastic container and mixed with 4 ml of saline. The container was capped and the contents were shaken until a substantially homogenous paste of uniform consistency was obtained. The paste was formed into a cylindrical shape and placed into a 10cc BD polypropylene disposable luer syringe. A second 10cc BD luer syringe containing 3ml of nitrogen then was connected to the syringe containing the paste such that the paste could be passed from syringe to syringe. The paste and gas were extruded back and forth between the syringes to thoroughly mix and disperse the gas throughout the paste until a substantially homogeneous foam-like composition of

uniform consistency was obtained. The syringes were then capped and five of the filled syringes were sterilized by irradiation at a dose of 25 kGy. The Peak Expression Force was determined and presented in Table 1. Unsterilized samples are designated as 1a' and sterilized samples are designated as 1b'.

Example 2:

A total of ten samples were prepared as follows. A saline solution containing 0.005% by weight of benzalkonium chloride and 5% weight of glycerol was prepared. This solution was used to prepare homogenous gelatin-powder pastes as described in Example 1. The paste was formed into a cylindrical shape and placed into a 10cc BD polypropylene disposable luer syringe. The syringes were then capped and five of the filled syringes were sterilized by irradiation at a dose of 25kGy. The Peak Expression Force was determined and presented in Table 1. Unsterilized samples are designated as 2a and sterilized samples are designated as 2b.

A total of ten samples were prepared as follows. A saline solution containing 0.005% by weight of benzalkonium chloride and 5% by weight of glycerol was prepared. This solution was used to prepare homogenous gelatin-powder pastes as described in Example 1. A second 10cc BD luer syringe containing 3ml of nitrogen then was connected to the syringe containing the paste such that the paste could be passed from syringe to syringe. The paste and gas were extruded back and forth between the syringes to thoroughly mix and disperse the gas throughout the paste until a homogeneous foam-like composition of uniform consistency was obtained. The syringes were then capped and five of the filled syringes were sterilized by irradiation at a dose of 25kGy. The Peak Expression force was determined and presented in Table 1. Unsterilized samples are designated as sample 2a' and sterilized samples are designated as 2b'.

Table 1

Samples	Peak Expression Force lbs (n=5)
Samples 1a	21.8
Samples 1b	26.4
Samples 1'a	12.0
Samples 1'b	21.0
Samples 2a	17.2
Samples 2b	22.4
Samples 2'a	11.8
Samples 2'b	16.8

As the data in Table 1 indicates, the inclusion of the gaseous phase homogenously dispersed throughout the paste significantly reduces the peak expression force of the composition prior to sterilization compared to pastes that do not include the homogenously dispersed gaseous phase or other additives. Consequently, the sterilized composition including the homogenously dispersed gaseous phase exhibits an expression force significantly less than that of a sterilized paste that does not include the homogenously dispersed gaseous phase. In fact, the sterilized composition including the gas phase approximates the expression force of the pre-sterilized paste containing no gaseous phase or additives. Thus, a fully sterilized composition may be provided with flowability and/or injectability, as evidenced by peak expression force, equal to or better than that of a unsterilized paste containing no gaseous phase or additives, which is beneficial to health care providers at the point of use. The use of

additives, e.g. benzalkonium chloride and glycerol, may be used to further enhance the properties of the compositions of the present invention upon sterilization.

Example 3:

25 grams of Surgifoam® gelatin powder were mixed with 125 ml of normal saline containing 0.005% benzalkonium chloride and 5 % glycerol, based on weight of saline, until a uniform paste was formed. The resulting paste was loaded into a ½ pint Donvier mixer fitted with a mixing paddle. A tube connected to a nitrogen source was fitted through the lid of the mixer and the system "closed" to the environment by wrapping in a film. The system was purged with nitrogen for 20 minutes. The paste was then mixed to homogenously incorporate the nitrogen by rotating the paddle rapidly by hand. Mixing was terminated when the composition filled the available volume, indicating homogeneous distribution of the gas phase. The composition was loaded into a 60cc syringe and subsequently dispensed into 10cc BD luer syringes via a two-way luer connector. The density of the composition was approximately 0.7-0.75 grams/ml. The syringes were then capped and some of the filled syringes were sterilized by irradiation at a dose of 25kGy.

Example 4:

2.5 liters of normal saline containing 0.005% benzalkonium chloride and 5 % glycerol dissolved therein, based on the weight of saline, were added to a 2-gallon double planetary Ross mixer and mixed at maximum speed with a first portion of nitrogen for 5 minutes to form a foamed liquid. 500 grams of Surgifoam gelatin powder and the balance of nitrogen were added to the foamed liquid over a 12-minute time period with continuous mixing. The composition was mixed for a further 10 minutes

after all of the powder and gas was added. The density of the resulting composition was 0.6 grams/ml. The composition was dispensed into 12cc Monoject syringes .

Example 5:

One-gram samples of Surgifoam® gelatin powder each were mixed with 5 ml of a saline solution containing 0.005% benzalkonium chloride and 5 % glycerol to form uniform pastes. The resulting paste was back-loaded into 10cc BD luer syringes. All air was extruded from the syringes, leaving the paste packed in the syringe. The first set of syringes was irradiated with no gas incorporated therein and designated as sample 5a. A second set of samples was prepared by dispensing 3ml of nitrogen into the syringes containing the uniform paste. The syringes were capped without further mixing and then stored at 4°C. The samples were designated as samples 5b. The third set of samples were prepared by extruding the paste back and forth between the first syringe and a second syringe containing 3 ml of nitrogen until all of the nitrogen was homogenously incorporated into the paste. The fill-volume of the resulting homogeneous compositions was approximately 9 ml and the density of the composition was approximately 0.7 grams/ml. The syringes were then capped and some of the pre-filled syringes were sterilized by irradiation at a dose of 25kGy. The Peak Expression Force of the three sets of samples was determined and presented in Table 2.

TABLE 2

Samples	Peak Expression Force lbs (n=5)
Sample 5a	21.7
Sample 5b	20.7
Sample 5c	15.5

As the data in Table 2 indicates, homogeneous distribution/dispersion of the gas throughout the paste is essential to reduce the peak expression force of the composition prior to irradiation and to maintain the lower peak expression force of the composition after irradiation, compared to pastes containing no gas or having gas poorly or partially dispersed there through.

Example 6:

One gram of Surgifoam® gelatin powder was mixed with 5 ml of normal saline to form a uniform paste. The resulting paste was back-loaded into a 10cc BD luer syringe. All air was extruded from the syringe leaving the paste packed in the syringe. A second set of 10cc syringes containing nitrogen with volume ranging from lml to 4ml , respectively, was fitted to the first via a two-way luer connector. The paste was extruded into the gas and then passed back and forth between the two syringes until all of the gas was homogeneously incorporated into the paste. The fill-volume of the resulting composition was approximately 6-10 ml and the density was approximately 0.60 to 1.0 grams/ml, each depending on the volume of gas introduced into the paste. The syringes were then capped and some of the pre-filled syringes were sterilized by irradiation at a dose of 25kGy. Sterilized samples were noted as samples 6a through

6e, respectively. The Peak Expression Force of the sterilized samples was determined and presented in Table 3.

Table 3

Samples	Gas Volume (ml)	Density (g/ml) (Pre-sterilized)	Peak Expression Force lbs n=5
Sample 6a	0	1.00	14.8
Sample 6b	1	0.86	12.9
Sample 6c	2	0.75	10.6
Sample 6d	3	0.66	8.6
Sample 6e	4	0.60	8.0

Example 7:

One gram of Surgifoam gelatin powder was mixed with 5 ml of normal saline to form a uniform paste. The resulting paste was back-loaded into a 10cc BD luer syringe. All air was extruded from the syringe, leaving the paste packed in the syringe. A second set of 10cc syringes containing air with volume ranging from 0ml to 4 ml, respectively, were fitted to the first syringe via a two-way luer connector. The paste was extruded into the gas and then passed back and forth between the two syringes until all of the gas was homogenously incorporated into the paste. The fill-volume of the resulting composition was approximately 6-10 ml and the density was approximately 0.60 to 1.0 grams/ml, each depending on the volume of gas introduced into the paste. The syringes were then capped and some of the filled syringes were sterilized by irradiation at a dose of 25kGy. Sterilized samples were noted as samples

7a through 7e, respectively. The Peak Expression Force of the sterilized samples was determined and presented in Table 4.

Table 4

Samples	Gas Volume (ml)	Density (g/ml) (Pre-sterilized)	Peak Expression Force lbs n=5
Sample 7a	0	1.00	14.8
Sample 7b	1	0.86	11.0
Sample 7c	2	0.75	10.9
Sample 7d	3	0.66	10.1
Sample 7e	4	0.60	10.0

Example 8: Hemostatic performance of different materials in porcine splenic biopsy punch model

A porcine spleen biopsy punch model was used for evaluation of the hemostatic properties of samples prepared in Examples 1 through 7 and 9. A 6-mm biopsy punch was used to cut a tissue flap 3mm deep. The tissue flap was cut out and 0.4 ml of the test materials was applied to the wound site. Manual compression was held over the wound site for 2 minutes. The wound site was then observed for up to 3 minutes for signs of bleeding. If bleeding was observed, additional applications of manual compression for 30 seconds each time were used until complete hemostasis was achieved. Table 5 lists the results of the evaluation. Results for unsterilized or sterilized samples are represented as an average values for all samples tested.

TABLE 5

In vivo Hemostasis Performance

Samples	Number of Compressions	Time to Hemostasis (mins:seconds)
Samples 1a	3	3:35 (n=2)
Samples 2a	3	3:33 (n=2)
Samples 1b	1	2:00 (n=3)
Samples 2b	2	3:00 (n=6)

Example 9:

Two vials of lyophilized Bovine thrombin (20,000 units Thrombogen JJMI) were reconstituted in 20 ml of saline to provide a working solution of 1000 u/ml. Clotting activity was measured in an *in vitro* test as described in Example 10. One vial of this material was stored at 4-8°C and the clotting activity measured at day 1, day 8 and day 30, respectively. The second vial was sterilized by gamma irradiation (25kGy) and the clotting activity measured as above. The unsterilized and sterilized samples were designated samples 9a and 9b, respectively. Both sterilized and unsterilized samples were stored at 4-8 °C between measurements.

Another 2 vials of 20,000 units of lyophilized bovine thrombin were reconstituted in saline containing 0.005% benzalkonium chloride and 5% glycerol. One vial was stored at. 4-8°C and the clotting activity was measured at day 0, day 1, day 8 and day 30. The second vial was sterilized by gamma irradiation (25kGy) and the clotting activity measured as above. In between measurements both the sterilized and unsterilized samples were stored at 4-8 °C. The unsterilized and sterilized samples were designated samples 9c and 9d, respectively.

Several samples of gelatin paste containing the thrombin noted above were prepared by mixing 1 gram of Surgifoam gelatin powder with 5 ml of thrombin solution. The resulting paste was loaded into a 10cc syringe. Samples were then either sterilized at 25kGy followed by storage at 4-8°C, or stored unsterilized at 4-8°C. Samples so prepared are designated and identified below.

Sample 9e = 1 g Surgifoam® powder plus 5 ml of sample 9a; Sterilized
Sample 9f = 1 g Surgifoam® powder plus 5 ml of sample 9a plus 3 ml Nitrogen: Foamed and Sterilized
Sample 9g = 1 g Surgifoam® powder plus 5 ml of sample 9c; Unsterilized
Sample 9h = 1 g Surgifoam® powder plus 5 ml of sample 9c; Sterilized
Sample 9i = 1 g Surgifoam® powder plus 5 ml of sample 9c plus 3 ml Nitrogen; Foamed and Sterilized

Example 10:

Measurement of Thrombin activity by an *in vitro* coagulation test in a Fibrometer instrument (BBL)

Method: Serial dilutions of test sample containing thrombin were prepared in Veronal buffer pH 7.2. 0.2 ml of pooled normal plasma (Citrol Level 1 control plasma-Dade Diagnostics) was warmed to 37°C in the fibrometer incubator block. 0.1 ml of pre-warmed sample dilution was added to the plasma and the timer started simultaneously. The time to clot formation was recorded. All samples were tested in duplicate and an average clotting time calculated. Data was graphed as the $\log_{10}$ dilution vs. $\log_{10}$ clotting time and a regression analysis performed. Freshly prepared thrombin was considered to have 100% activity and all other samples were calculated

as a percentage of the activity relative to the freshly prepared thrombin. Results are presented in Table 6 and Table 7.

TABLE 6

Effect of Storage time on Thrombin Activity:

Stabilization by Formulated Gelatin Paste

Storage Solution (Stored at 6°C)	Percent Loss in Thrombin Activity			
	Time 0	Day 1	Day 8	Day 30
9a	0	0	53.3	90.8
9c	0	NA	41.1	82.9
9g	0	0	0.8	0

TABLE 7

Effect of Gamma Irradiation on Thrombin Activity:

Stabilization by Formulated Gelatin paste

Media for Sterilized Thrombin * Samples (5ml/g gelatin powder- 25 kGy Dose)	% Loss in Thrombin Activity	
	Day 6	Day 20
9b	100	100
9d	96.0	100
9e	72.6	NA
9f	66.8	56-72
9h	79.2	ND
9i	63.8	61-73

TABLE 8

***In vivo* Hemostasis Performance of Pre-filled Thrombin/Gelatin Paste**

TIME: SAMPLE	Number of Compressions	Time to Hemostasis (mins:secs)
Day 0: 9g	1	0:30
Day 42: 9g	1	0:30
Day 42: 9g	1	0:30
Day 42: 9h	1	0:30
Day 42: 9h	1	0:30